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Trace elements' reference levels in blood of breeding black-browed albatrosses *Thalassarche melanophris* from the Falkland Islands

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Abstract

Trace elements' concentration in the ocean is fast growing and is a source of major concern. Being charismatic and at the top of food chains, seabirds are often used as biological monitors of contaminants. We studied the concentration of trace elements in blood of black-browed albatross from the Falklands Islands, which we here show, by tracking with Geolocators, forage over most of the Patagonian Shelf. Levels of trace elements were measured in males and females from two different islands. Blood concentrations of trace elements were not significantly different between islands, which is consistent with observations from foraging behaviour revealing that birds from both islands foraged in broadly the same areas in the months before sampling. Arsenic and selenium concentrations in females were higher than in males. Sex-related differences in the concentration of these elements may be related to unknown slight differences in diet or to differences in assimilation between sexes. These results provide reference values for monitoring elemental contamination in the Patagonian Shelf Marine Ecosystem using black-browed albatrosses, one of the most abundant top predators and a suitable sentinel for the region's environmental health.

Keywords: *Thalassarche melanophris*. Trace elements. Sex. Foraging behaviour. Patagonian Shelf

Introduction

Contamination by trace elements is growing at a fast pace and is an issue of major concern for the health of the environment and organisms (Scheuhammer 1987; Sánchez-Virosta et al. 2015;Evers 2018; Preeti et al. 2018). Plants and animals often assimilate and bioaccumulate trace elements in a predictable manner and therefore some taxa have been successfully used as biological monitors of elemental pollution in marine and terrestrial ecosystems (Anderson et al. 2010; Yildirim and Sasmaz 2017; Furtado et al. 2019).

Several trace elements (cobalt, copper, manganese, selenium, and vanadium) are essential components of physiological and biochemical processes of organisms (e.g. vertebrates), but beyond certain levels of concentration these elements also become toxic (Abdulla and Chmielnicka 1990; Senesi et al. 1999; Bansal and Asthama 2018) Others, such as arsenic, cadmium, mercury, and strontium , are poorly tolerated by the organisms and are usually considered as a non-essential elements for life and are known to be toxic even at low concentration levels (Naidu et al. 1999; Senesi et al. 1999; Bansal and Asthama 2018). Arsenic is considered as one of the most highly toxic and carcinogenic elements by the US Environmental Protection Agency (EPA) (ATSDR 2020).

The Patagonian Shelf Large Marine Ecosystem, in the Southern Ocean, is one of the most productive marine areas worldwide (Croxall and Wood 2002). Here currents and mixing, including localised upwelling, contribute to resuspension of sediments and organic particles, resulting in a natural redistribution of trace elements, notably those behaving like nutrients (e.g. copper and selenium) (Orren and Monteiro 1985; Cutter and Cutter 1995; Zhang et al. 2014; Gworek et al. 2016). Elements such as mercury are massively transported by the global atmospheric circulation and other such as cadmium, cobalt, lead and manganese, are distributed through oceanic circulation. Such inflows to what is present in the Southern Ocean determine the concentration of elements in this marine ecosystem (Chester 1990; Gaiero et al. 2003; Bargagli 2008; Cossa et al. 2011). Moreover, several anthropogenic sources (sewage effluents, agricultural runoff, oil extraction and transportation and mining) contribute to contamination by metals and metalloids in the ocean, including water and sediments (Gaiero et al. 2002).

The distribution of trace elements in marine waters is complex (Boye et al. 2012). Trace elements are not distributed uniformly in the ocean, and their concentration vary among areas as well as vertically within the water column (Cutter and Cutter 1995; de Villiers 1999; Bown et al. 2011; Cossa et al. 2011; Boye et al. 2012; Heller and Croot 2015; Schlesinger et al. 2017). Such variability in the distribution of trace elements causes different assimilation patterns within food webs (Santos et al. 2006). Moreover, elements such as cadmium, cobalt, copper, manganese, strontium and vanadium are accumulated by organisms of different trophic levels, but do not biomagnify (even in top predators, such as seabirds) because biodilution processes increase significantly with trophic position (Campbell et al. 2005; Nfon et al. 2009; Konovalenko et al. 2016; Liu et al. 2016, 2017, 2019; Signa et al. 2019). Conversely, the extent of arsenic and selenium biomagnification through the food web is a complex issue and is still a matter of scientific debate (Campbell et al. 2005; Mathews and Fisher 2008; Nfon et al. 2009; Stewart et al. 2010; Huang 2016; Furtado et al 2019). Selenium is transferred in food chains, and biomagnification does occur (Rainbow 2018). Stewart et al (2010) proposed a "trophic transfer function" to describe increased selenium concentrations in marine food webs, because transfer from prey to predator is dependent on concentration in a non-linear manner. Arsenic, usually in its inorganic forms, decrease with increasing trophic levels, but organic arsenic, especially arsenobetaine, increases (see review Huang 2016). The behaviour of mercury is well-known, and it biomagnifies through the food chains, thus top predators generally show higher mercury levels than primary producers (Santos et al. 2006).

Seabirds are top marine predators known to accumulate a variety of trace elements present in marine environment (Anderson et al. 2010; Tavares et al. 2013; Carravieri et al. 2014, 2018). Those are accumulated in different tissues such as blood, feathers, liver, kidney and muscle (Anderson et al. 2009; Aazami et al. 2011). Blood has been widely used to assess medium- to short-term variations in element concentrations of trace elements in the environment, reflecting accumulation through diet over approximately one to two months preceding sampling in seabirds (Hobson and Clark 1992, 1993; Carleton and Martínez del Rio 2005; Zanden et al. 2015). This period vary among taxa according to metabolism and it can be higher in some other vertebrates such as reptiles (Rosenblatt and Heithaus 2013).

The Falkland Islands are located in the Southwest Atlantic Ocean, where the level of trace elements bioavailability is poorly documented (Furtado et al. 2019). Black-browed albatrosses *Thalassarche melanophris* from the Falkland Islands mostly forage in the Subantarctic Zone, over the rich waters of the Patagonian Shelf (Granadeiro et al. 2017; Ponchon et al. 2019). Furthermore, birds are relatively sedentary, remaining in the Patagonian shelf and shelf-break also during the non-breeding season (Ponchon et al. 2019). Globally, the species is currently classified as Least Concern according to the IUCN (IUCN, 2020).

The levels of bioaccumulation of trace elements are important indicators of environmental quality and health of seabirds (Nicholson and Osborn 1983; Anderson et al. 2010; Sánchez-Virosta et al. 2015; Evers 2018). Seabirds are generally abundant at their nesting colonies, forage over wide oceanic areas, they are often easy to capture and can be sampled with minimal disturbance. Blood is a good tissue to monitor levels of several trace elements over the Patagonian Shelf Large Marine Ecosystems during the breeding season. An estimated 67.0% - 70.0% of the global population of this albatross species nests in the Falkland Island (ACAP 2009), with a total population in excess of 475,500 pairs (Wolfaardt 2013). Being well-known charismatic top predators in Patagonian Shelf ecosystem, black-browed albatrosses have the potential to be used as biological monitors and to communicate environmental issues to decision-makers and the general public.

The goal of this research was to establish baseline levels of trace elements in blood of black-browed albatrosses, as samplers of the Patagonian Shelf Large Marine Ecosystem, during the breeding period . We determined trace elements concentrations in blood samples of birds from Beauchene and New Island, two islands located in the South and West of the Falkland Islands, respectively, roughly reflecting accumulation between October and December 2016. We also assessed the spatial distribution of birds from both islands during this period. Finally, we tested whether there were differences in the accumulation of trace elements among sexes, as found in other waterbirds and in albatrosses for cadmium (e.g. Carravieri et al. 2014), mercury (e.g. Tavares et al. 2013) and selenium (e.g. Wilson et al. 2004).

Methods

Study sites and trace elements concentration survey

Blood sampling was carried out during December 2016 and early January 2017. We collected a small amount of blood (< 2.0 ml) from 20 adult black-browed albatrosses breeding on New Island (51°43'S, 61°19'W) and 18 birds from Beauchene Island (52°50' S, 59°10' W). We took a small aliquot of each sample into a different container for sex determination using molecular methods. The sampling procedure took less than 5 minutes to complete, and all birds resumed their incubation and brooding duties after being released at the nest.

Samples for elements determination were lyophilized during 48 h and ground to powder for homogenization, before digestion. All samples were digested following an adaptation of the EPA method 3051A (Link et al. 1998). Blood samples were digested using a CEM MARS 5, model 240/50 microwave digestion system, with continuous temperature and pressure monitoring in a tightly closed vessel. Closed vessels also minimize potential losses of analytes due to volatilization (Link et al. 1998). Previously, PFA vessels were decontaminated in the microwave system using 3.0 ml of HNO_3, and then rinsed with ultrapure water and air-dried. Approximately 50.0 mg (overall range: 4.1 – 190.3 mg) of sample were weighted in PFA digestion vessels and subsequently we added 0.4 mL of nitric acid (HNO_3). The microwave was programmed for 5 minutes, at 1600 W and 165 ºC, followed by 5 minutes at 1600 W and 190 ºC. The PFA vessels were then placed in a plate heater, and the contents evaporated to near dryness at 150 ºC. Finally, the residue was dissolved in 0.4 mL of hydrogen peroxide (H_2O_2), and the solutions heated for 30 minutes, at 150 ºC. After cooling at room temperature, the solutions were transferred into 10.0 mL volumetric flasks and then filled with ultrapure water (MQ filtration system) to the 10.0 mL and then analysed by high resolution ICP-MS (Thermo Elemental, X-Series). The experimental conditions for ICP-MS analysis were as follows: forward power 1400 W, peak jumping mode, 150 sweeps per replicate, dwell time 10 minutes and dead time 30 minutes. The polyatomic and isobaric interferences were minimized by setting the ratios $^{137}Ba^{2+}/^{137}Ba$ and $^{140}Ce^{16}O/^{140}Ce$ to 0.02, under routine operating conditions. The internal standard was ^{115}In. For quality control, the certified reference material NIST-SRM 2976 (mussel tissue) and TORT-2

(Lobster Hepatopancreas) were analysed simultaneously (see results in Supplementary Table A1).

Among trace elements, arsenic, mercury, selenium, and strontium were quantified in all individuals, while cadmium, cobalt, manganese and vanadium could only be detected in a few black-browed albatrosses. The limit of quantification (LoQ), was calculated as ten times the standard deviation (SD) of the blank divided by the slope of the analytical curve, respectively (Taleuzzaman 2018), expressed in concentration (ug/L). The limit of quantification was converted to mg/kg dry weight per sample (Supplementary Table A2 and Supplementary Table A3).

Sex determination

Black-browed albatrosses exhibit a clear sexual dimorphism in some behaviours and in the present work, 12 birds were sexed by direct observation of the pair during the pre-laying period. Whenever this was not possible, we used the molecular-based method described in Fridolfsson and Ellegren (1999) using DNA extracted from blood samples (n = 26).

Geolocation data

In order to obtain information on the areas used by albatrosses prior to the collection of our samples, we deployed leg-mounted Mk19 light-level geolocators (GLS) on 20 adult black-browed albatrosses on Beauchene Island in December 2015, which were then recovered in January 2017. Of these, 12 loggers delivered usable data. Positions were obtained from light information analyzed following the method employed by Dias et al. (2011) and Phillips et al. (2004). GLS data of black-browed albatross from New Island were not available for 2016, and therefore we used GLS information from 7 adult birds collected in 2013. GLS positions obtained between October and December (e.g. 3 months prior to sample collection) were examined using Kernel Density Estimate (KDE) analysis, which were used to depict 50.0%, 75.0% and 90.0% of density contour areas (the estimated foraging range). All calculations were carried out with the adehabitatHR package (Calenge 2006) running in R (R Core Team 2019).

Data analysis

Trace elements levels with concentrations lower than limit of quantification in more than 50% of the individuals, which included cadmium, cobalt, manganese and vanadium, were excluded from subsequent statistical analyses (e.g. Anderson et al. 2010). The effects of island and sex in levels of trace elements were evaluated by two-way ANOVAs, after checking for data normality. Covariance models (ANCOVA) were used to test the effect of sex in the correlations between trace elements concentrations. Means are presented with standard deviations.

Results

Concentration of trace elements in blood

Levels of arsenic, cadmium, cobalt, copper, manganese, mercury, selenium, strontium and vanadium in blood of black-browed albatross are shown in Table 1.

We did not find any differences between islands in any of the trace elements analyzed. Conversely, there were significant differences between sexes (Fig. 2) in the concentrations of arsenic (Two-way ANOVA, effect of sex: $F_{1,34} = 7.56$, $p = 0.009$) and selenium ($F_{1,34} = 8.83$, $p = 0.005$) , with males showing lower concentrations than females (arsenic: females: 3.02 ± 0.97 mg/kg, males: 2.37 ± 0.49 mg/kg; selenium: females: 52.50 ± 24.13 mg/kg, males: 33.94 ± 13.43 mg/kg).

The analysis of covariance showed a significant correlation between the levels of selenium and arsenic ($F_{1,35} = 99.5$, $p < 0.001$), but there was no effect of sex ($F_{1,35} = 1.46$, $p = 0.234$). The same was observed in the relationship between copper and strontium ($F_{1,34} = 61.4$, $p < 0.001$, no effect of sex, $F_{1,34} = 0.48$, $p = 0.496$) and selenium and copper ($F_{1,34} = 4.67$, $p = 0.038$, no effect of sex, $F_{1,34} = 0.03$, $p = 0.864$).

The analysis of covariance revealed differences among sexes in the correlation between arsenic and copper ($F_{1,34} = 4.40$, $p = 0.043$), with concentrations of arsenic in females being ca. 0.6 mg/kg higher than in males, for the same values of copper ($F_{1,34} = 6.81$, $p = 0.013$). The same was observed for the relationship between selenium and mercury

($F_{1,35}$ = 5.62, p = 0.023), with concentrations of selenium in females being ca. 18.6 mg/kg higher than in males for the same values of mercury($F_{1,35}$ = 10.52, p = 0.002).

Discussion

Birds tracked in this study carried out foraging trips within the Patagonian shelf. More specifically, kernel density distributions of black-browed albatrosses from New Island and Beauchene Island (islands located 200 km apart) revealed no major differences in the areas of the Patagonian Shelf used between October and December (Fig. 1). Despite the high overlap between the kernels (ca. 62.0%), birds from New Island also seem to be using an area slightly more to the north than the one used by those from Beauchene Island. The levels of trace elements measured in black-browed albatross blood in those two different islands should be indicative of contamination in this broad Patagonian Shelf region (Table 1). The mean concentration of trace elements decreased in the following order: selenium > arsenic > copper > mercury > strontium > vanadium > manganese > cadmium > cobalt. Levels of contaminants in the Falkland Islands were generally low when compared with data from other species and locations in the Southern Ocean. Arsenic was the exception, as birds from the Falkland Islands showed comparatively high concentrations of this element (Supplementary Table A4).

We did not find any difference between islands in the levels of arsenic, copper, mercury, selenium and strontium in the blood of albatrosses (Table 1), which fits well with the large overlap in foraging areas of birds from the two colonies. The diet of albatross from New Island are broadly composed of fish (mostly *Sprattus fuegensis* and *Patagonotothen* sp.), Crustacea (mostly lobster krill *Munida gregaria*) and Scyphozoa (e.g. jellyfish) (McInnes et al. 2017a, b) and Thompson (1992), reported the same groups of prey for albatrosses from Beauchene Island, which again concurs with a high overlap in feeding areas and similar levels of contamination. Furtado et al. (2019) reported no significant differences in mercury levels in feathers of black-browed albatross between New Island and Beauchene Island.

In the ocean, arsenic, cadmium, copper, mercury, selenium and strontium occur in lower concentrations at the surface (< 100 m) than in deeper waters (Westerlund and Ghman

1991; Cutter and Cutter 1995; de Villiers 1999; Cutter et al. 2001; Heller and Croot 2015). Methylmercury concentration increases with depth to an intermediate maximum in the mesopelagic domain (Cossa et al. 2011). The concentration of copper, mercury and strontium appears to be higher in the Antarctic waters than in the other oceanic areas (Cossa et al., 2011; de Villiers, 1999; Heller and Croot, 2015). Furthermore, Antarctic Intermediate Waters exhibit some of the highest arsenic concentration observed in the ocean (Santosa et al. 1994; Cutter et al. 2001). For example, *Euphausia superba*, a commonly found prey in the diet of black-browed albatross from South Georgia, has 5.5 ± 1.1 mg/kg of selenium and 0.01 ± 0.01 mg/kg of mercury (Anderson et al. 2009, 2010). Recently Sontag et al. (2019), reported concentration of methylmercury between 0.00074 to 0.00294 mg/kg and 0.00026 to 0.00161 mg/kg in juvenile and adult *Euphausia superba* respectively. Besides contrasting levels of background contamination, differences in the food web structure may help explain the higher concentrations of several trace elements in black-browed albatrosses in South Georgia, compared to the Falkland Islands (Supplementary Table A4). In the former region, albatrosses forage mostly over deep oceanic waters (e.g. Wakefield et al. 2012) and will have a different exposure compared to those foraging around the Falkland Islands, where the birds forage almost exclusively over shallower environments on the shelf (e.g. Granadeiro et al. 2017; Ponchon et al. 2019).

The concentration of arsenic and selenium were significantly higher in females than in males (Fig. 2, Table 1). Males and females black-browed albatrosses from the Falklands show similar foraging areas year-round, and particularly during October-December (Ponchon et al. 2019; own unpublished data). Furthermore, they seem to have similar diets as assessed by nitrogen and carbon stable isotopes (Campioni et al. 2016). Hence, one could expect that element levels would be lower for females than males, because females have the possibility to eliminate some contaminants through excretion in eggs (Monteiro and Furness 1995; Kubota et al. 2002; Ackerman et al. 2016) . However, arsenic and selenium levels were higher in females than in males, suggesting that maternal transfer of these elements to the egg may be limited in this species. Metabolic differences between sexes and/or different assimilation rates may explain the lower levels of arsenic and selenium found in males. There have been previous studies that reported lower levels of arsenic, mercury and selenium in males than females (Becker et al. 2002; González-Solís et al. 2002; Wilson et al. 2004; Taggart et al. 2006; Lucia et al.

2010; Carvalho et al. 2013; Tavares et al. 2013; Carravieri et al. 2014; Ackerman et al. 2016). However, this pattern is dependent on the species of birds and/or location. Bustamante et al. (2016), have reported a difference in mercury, between female and male wandering albatrosses *Diomedea exulans* from South Georgia and suggested that maternal transfer of mercury to the egg was limited in this species. Pon et al. (2011) and Tavares et al. (2013) have reported no effect of sex in cadmium, cooper and mercury in feathers of albatrosses. However, the significance of the presence of elements such as cadmium in feathers is more difficult to assess, as cadmium does not seem to integrate this biological matrix in response to exposure levels.

We found a positive correlation between levels of mercury and of selenium in blood of black-browed albatrosses. This relationship is important, since selenium interacts with mercury to form inert complexes of high molecular weight in the blood, especially in the erythrocytes, and forms harmless non-diffusible complexes (mercury-selenium) in the liver and kidney and therefore acts as a form of protection against methylmercury toxicity (thus delivering a "detoxifying" effect for methylmercury) (Cuvin-Aralar and Furness 1991; Imura and Naganuma 1991; Yang et al. 2008). Some researchers have reported that mercury concentrations in feathers, liver and kidney of birds between 5 and 40 mg/kg can have negative impacts expressed by changes in reproduction and survival (Wolfe et al. 1998; Evers, 2008; Whitney and Cristol 2017). Overall, mercury levels in blood of black-browed albatross analyzed in this study are below the 5 mg/kg threshold in 97 % of the individuals sampled (n = 38), and the highest value recorded in our sample was 5.19 mg/kg. Most researchers investigating relationships between blood mercury and breeding and/or survival have found no suggestion for an impact of mercury in seabirds, which may indicate that seabirds exhibit extraordinary resistance to contamination (Goutte et al. 2014, 2015; Pollet et al., 2017; Bond et al. 2015; Tartu et al. 2016; Carravieri et al. 2018).

In our albatross samples, copper was correlated with strontium, arsenic and selenium and arsenic with selenium. Recent research suggests selenium antagonizes the toxicity of arsenic mainly through sequestration of this element into biologically inert arsenic - selenium compounds and/or through increasing the arsenic methylation capacity in the body (decreased tissue accumulation of arsenics and its toxic effects) in diverse organs and tissues (see review Zwolak 2020). These correlations between elements suggests an interaction between elements in the blood or, alternatively, an assimilation from a common source of pollution (e.g. foraging area or type of prey), but further research is required to unveil the mechanisms behind such relationships. We also highlight that our study presents the first baseline data on the concentrations of cobalt in blood of black-browed albatross.

Conclusion

This study establishes baseline levels for nine trace elements in black-browed albatross of the Falkland Islands that will be useful for future monitoring under a scenario of rapid changes in the ocean biogeochemistry (Geibert 2018). Albatrosses from Falkland Islands are exposed to low-to-moderate concentrations of trace elements, as compared to other islands and species in the Southern Ocean (Supplementary Table A4). Results of trace element concentrations suggest that birds from different Falkland colonies are exposed to the same background levels, which reflects the large overlap in foraging areas. The concentrations of arsenic and selenium varied with sex, despite the fact that the foraging areas of males and females show almost complete overlap (Pochon et al. 2019; own unpublished data). Potential differences between sexes in diet and physiology deserve further investigation.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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Table 1 Trace elements concentrations (number of samples; mean ± SD, mg/kg (Range) in blood of black-browed albatross from the Falkland Islands. Two-way ANOVA were performed to compare trace elements concentrations between sexes and colonies. Significant statistics ($p < 0.05$) are highlighted **in bold**.

Trace elements	Colonies								Two-way ANOVA effect of sex	Two-way ANOVA effect of island
	Beauchene Island				New Island					
	Female		Male		Female		Male			
	Mean ± SD (Range)	Number of samples	Mean ± SD (Range)	Number of samples	Mean ± SD (Range)	Number of samples	Mean ± SD (Range)	Number of samples		
Arsenic (As)	2.8 ± 1.0 (1.9 – 4.8)	6	2.4 ± 0.6 (1.7 – 3.6)	12	3.2 ± 0.9 (2.1 – 4.2)	5	2.4 ± 0.4 (1.6 – 3.0)	15	$\mathbf{F_{1,34} = 7.56, p = 0.009}$	$F_{1,34} = 0.06, p = 0.81$
Cadmium (Cd)		0		0	0.1 ± 0.03 (0.01 – 0.1)	4	0.07 ± 0.1 (0.01 – 0.2)	3		
Cobalt (Co)		0		0		0	0.02 ± 0.02 (0.01 – 0.05)	3		
Cooper (Cu)	1.6 ± 0.4 (1.2 – 2.3)	6	1.5 ± 0.2 (1.3 – 1.8)	11	1.9 ± 0.2 (1.6 – 2.0)	5	1.6 ± 0.4 (1.3 – 2.9)	15	$F_{1,31} = 0.69, p = 0.41$	$F_{1,34} = 1.41, p = 0.24$
Manganese (Mg)		0	0.1	1	0.2 ± 0.1 (0.08– 0.4)	4	0.2 ± 0.2 (0.07– 0.9)	14		
Mercury (Hg)	1.3 ± 0.7 (0.4 – 2.2)	6	1.5 ± 1.3 (0.4 – 5.2)	12	1.9 ± 0.8 (1.1 – 3.1)	5	1.6 ± 0.6 (0.5 – 3.0)	15	$F_{1,34} = 0.002, p = 0.96$	$F_{1,34} = 0.78, p = 0.38$
Selenium (Se)	54.0 ± 31.5 (28.6 – 111.0)	6	32.3 ± 18.2 (15.1 – 70.7)	12	50.7 ± 14.5 (37.0 – 69.6)	5	35.3 ± 8.4 (19.6 – 47.5)	15	$\mathbf{F_{1,34} = 8.83, p = 0.005}$	$F_{1,34} = 0.004, p = 0.95$
Strontium (Sr)	0.9 ± 0.9 (0.4 – 2.6)	6	0.5 ± 0.3 (0.2 – 1.1)	12	1.2 ± 0.7 (0.6 – 2.2)	5	0.9 ± 0.9 (0.4 – 4.0)	15	$F_{1,34} = 2.93, p = 0.10$	$F_{1,34} = 1.55, p = 0.22$
Vanadium (V)		0		0	0.8 ± 0.1 (0.7 – 0.9)	2	0.7	1		

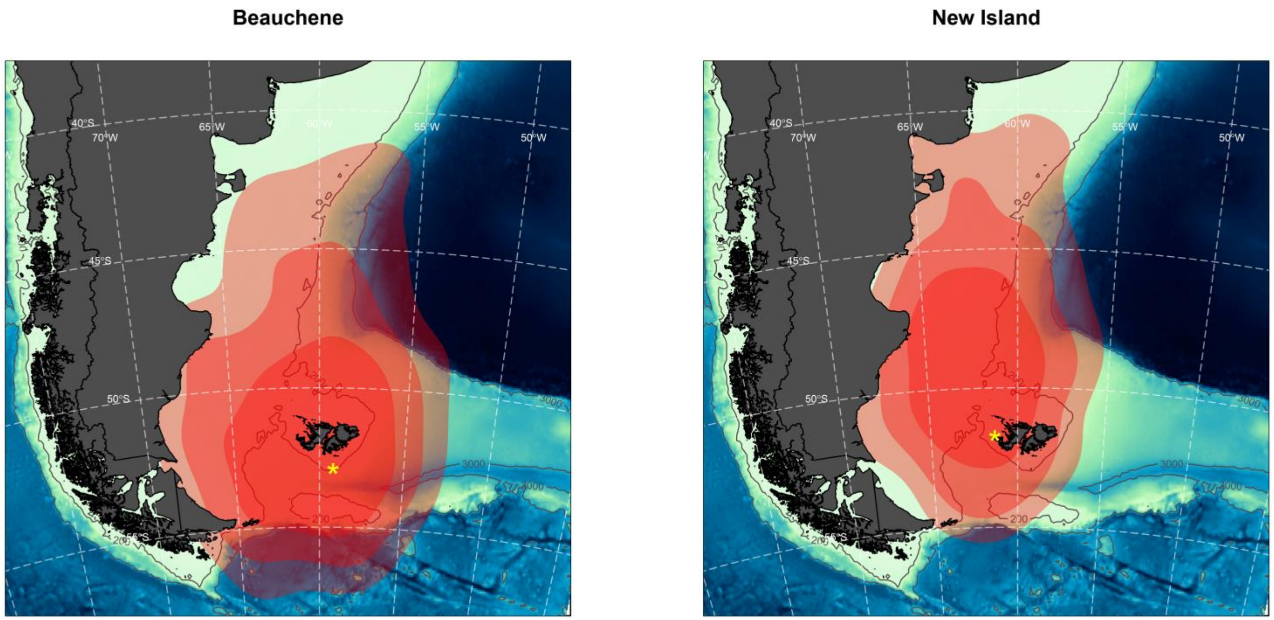

Fig. 1. Kernel density estimates of Black-browed albatrosses (BBA) successfully tagged with geolocators at Beauchene (2016, N=12) and New Island (2013, N=7) between October and December (incubation-early chick-rearing). Colors represent 50%, 75% and 90% contours.

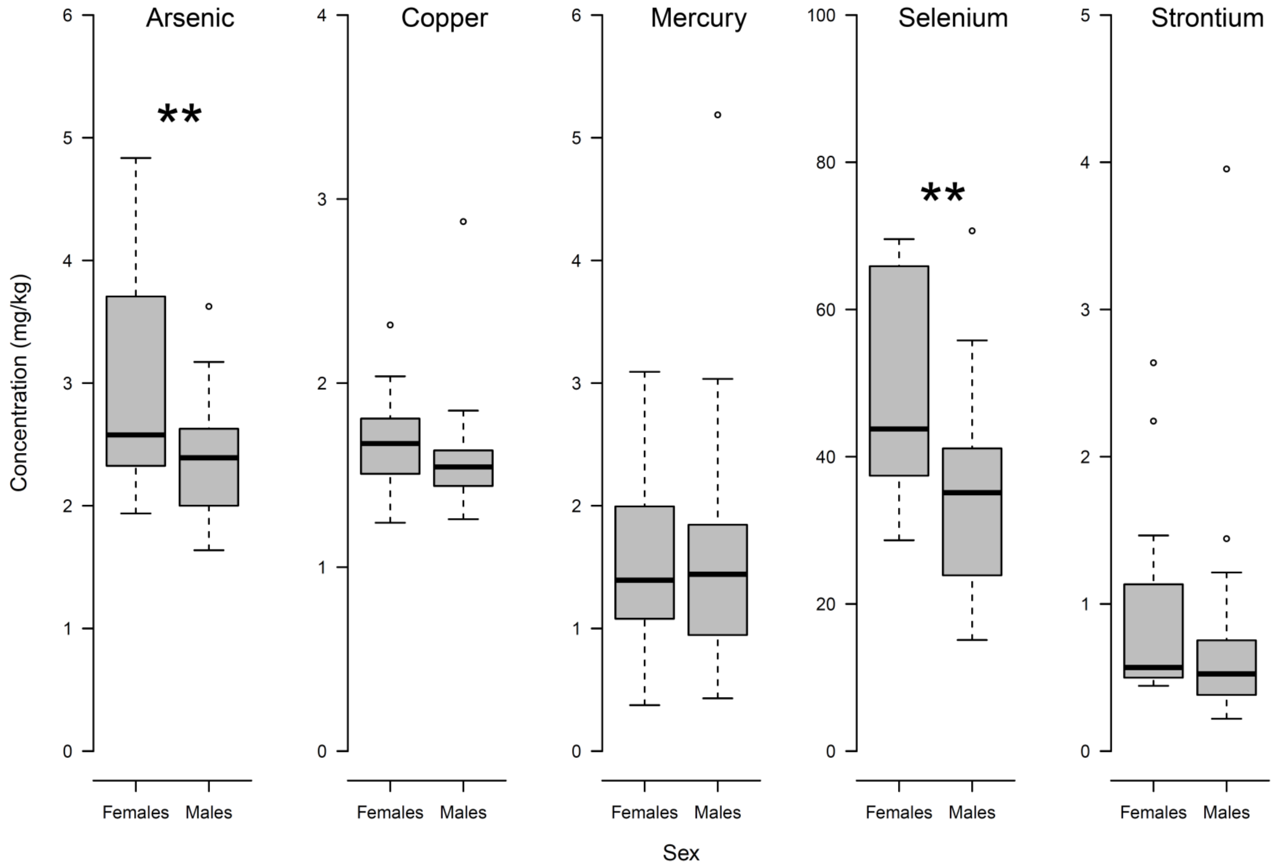

Fig. 2. Black-browed albatross blood concentrations (mg/kg) of five trace elements (arsenic, copper, mercury, selenium, and strontium) grouped by sex. Stars indicate statistically significant differences ($p < 0.05$). The box shows the inter-quartile range (25–75th percentile), the bold line is the median, the whiskers represent maximum and minimum, and (when present) dots indicate outliers.

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